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RAW SEQUENCE LISTING DATE: 03/05/2003
PATENT APPLICATION: **US/10/009,500B** TIME: 15:15:15

Input Set : **A:\Merck233.app**
Output Set: **N:\CRF4\03052003\J009500B.raw**

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 3 <110> APPLICANT: KORDOWICZ, MARIA
 4       GUESSOW, DETLET
 5       HOFMANN, UWE
 6       PACUSZKA, TADEUSZ
 7       GARDAS, ANDRZEJ
 9 <120> TITLE OF INVENTION: HYALURONIDASE FROM THE HIRUDINARIA MANILLENSIS,
10       ISOLATION, PURIFICATION AND RECOMBINANT METHOD OF
11       PRODUCTION
13 <130> FILE REFERENCE: MERCK 2332
15 <140> CURRENT APPLICATION NUMBER: 10/009,500B
16 <141> CURRENT FILING DATE: 2002-04-08
18 <160> NUMBER OF SEQ ID NOS: 20
20 <170> SOFTWARE: PatentIn Ver. 2.1
22 <210> SEQ ID NO: 1
23 <211> LENGTH: 488
24 <212> TYPE: PRT
25 <213> ORGANISM: Hirudinaria manillensis
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32              20                  25                  30
34 Lys Gly Leu Trp Ser Phe Val Asp Ile Thr Ser Pro Lys Leu Phe Lys
35          35                  40                  45
37 Leu Leu Glu Gly Leu Ser Pro Gly Tyr Phe Arg Val Gly Gly Thr Phe
38      50                  55                  60
40 Ala Asn Trp Leu Phe Phe Asp Leu Asp Glu Asn Asn Lys Trp Lys Asp
41  65                  70                  75                  80
43 Tyr Trp Ala Phe Lys Asp Lys Thr Pro Glu Thr Ala Thr Ile Thr Arg
44                  85                  90                  95
46 Arg Trp Leu Phe Arg Lys Gln Asn Asn Leu Lys Lys Glu Thr Glu Asp
47              100                 105                 110
49 Asp Leu Val Lys Leu Thr Lys Gly Ser Lys Met Arg Leu Leu Phe Asp
50          115                 120                 125
52 Leu Asn Ala Glu Val Arg Thr Gly Tyr Glu Ile Gly Lys Lys Met Thr
53      130                 135                 140
55 Ser Thr Trp Asp Ser Ser Glu Ala Glu Lys Leu Phe Lys Tyr Cys Val
56 145                 150                 155                 160
58 Ser Lys Gly Tyr Gly Asp Asn Ile Asp Trp Glu Leu Gly Asn Glu Pro
59                  165                 170                 175
61 Asp His Thr Ser Ala His Asn Leu Thr Glu Lys Gln Val Gly Glu Asp
62              180                 185                 190
64 Phe Lys Ala Leu His Lys Val Leu Glu Lys Tyr Pro Thr Leu Asn Lys
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65          195                 200                 205
67 Gly Ser Leu Val Gly Pro Asp Val Gly Trp Met Gly Val Ser Tyr Val
68      210                 215                 220
70 Lys Gly Leu Ala Asp Gly Ala Gly Asp Leu Val Thr Ala Phe Thr Leu
71 225                 230                 235                 240
73 His Gln Tyr Tyr Phe Asp Gly Asn Thr Ser Asp Val Ser Thr Tyr Leu
74                  245                 250                 255
76 Asp Ala Thr Tyr Phe Lys Lys Leu Gln Gln Leu Phe Asp Lys Val Lys
77              260                 265                 270
79 Asp Val Leu Lys Asn Ser Gln His Lys Asp Lys Pro Leu Trp Leu Gly
80          275                 280                 285
82 Glu Thr Ser Ser Gly Tyr Asn Ser Gly Thr Lys Asp Val Ser Asp Arg
83      290                 295                 300
85 Tyr Val Ser Gly Phe Leu Thr Leu Asp Lys Leu Gly Leu Ser Ala Ala
86 305                 310                 315                 320
88 Asn Asn Val Lys Val Val Ile Arg Gln Thr Ile Tyr Asn Gly Tyr Tyr
89                  325                 330                 335
91 Gly Leu Leu Asp Lys Asn Thr Leu Glu Pro Asn Pro Asp Tyr Trp Leu
92              340                 345                 350
94 Met His Val His Asn Ser Leu Val Gly Asn Thr Val Phe Lys Val Asp
95          355                 360                 365
97 Val Ser Asp Pro Thr Asn Lys Ala Arg Val Tyr Ala Gln Cys Thr Lys
98      370                 375                 380
100 Thr Asn Ser Lys His Thr Gln Ser Arg Tyr Tyr Lys Gly Ser Leu Thr
101 385                 390                 395                 400
103 Ile Phe Ala Leu Asn Val Gly Asp Glu Asp Val Thr Leu Lys Ile Asp
104                 405                 410                 415
106 Gln Tyr Gly Gly Lys Lys Ile Tyr Ser Tyr Ile Leu Thr Pro Glu Gly
107             420                 425                 430
109 Gly Gln Leu Thr Ser Gln Lys Val Leu Leu Asn Gly Lys Glu Leu Lys
110         435                 440                 445
112 Leu Val Ser Asp Gln Leu Pro Glu Leu Asn Ala Asn Glu Ser Lys Thr
113     450                 455                 460
115 Ser Phe Thr Leu Ser Pro Lys Thr Phe Gly Phe Phe Val Val Ser Asp
116 465                 470                 475                 480
118 Ala Asn Val Glu Ala Cys Lys Lys
119                 485
122 <210> SEQ ID NO: 2
123 <211> LENGTH: 1464
124 <212> TYPE: DNA
125 <213> ORGANISM: Hirudinaria manillensis
127 <220> FEATURE:
128 <221> NAME/KEY: CDS
129 <222> LOCATION: (1)..(1464)
131 <220> FEATURE:
132 <221> NAME/KEY: variation
133 <222> LOCATION: (667)..(669)
134 <223> OTHER INFORMATION: This codon codes for a Tyr or Asn
136 <400> SEQUENCE: 2
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RAW SEQUENCE LISTING DATE: 03/05/2003
PATENT APPLICATION: US/10/009,500B TIME: 15:15:15

Input Set : A:\Merck233.app
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    137 aaa gag att gcc gtg aca att gac gat aag aat gtg att gca tct gcc   48
    138 Lys Glu Ile Ala Val Thr Ile Asp Asp Lys Asn Val Ile Ala Ser Ala
    139   1               5                  10                  15
    141 agt ggg tct ttc ctt gga gtt gcc ttt gat gcg tct cta ttt tcg ccc   96
    142 Ser Gly Ser Phe Leu Gly Val Ala Phe Asp Ala Ser Leu Phe Ser Pro
    143             20                  25                  30
    145 aag ggt ctt tgg agc ttt gtt gat att acc tct cca aaa ttg ttc aaa   144
    146 Lys Gly Leu Trp Ser Phe Val Asp Ile Thr Ser Pro Lys Leu Phe Lys
    147         35                  40                  45
    149 ttg ctg gaa gga ctt tct cct gga tac ttc agg gtt ggc gga acg ttt   192
    150 Leu Leu Glu Gly Leu Ser Pro Gly Tyr Phe Arg Val Gly Gly Thr Phe
    151     50                  55                  60
    153 gcc aat tgg ctg ttt ttt gac ttg gac gaa aat aat aag tgg aag gat   240
    154 Ala Asn Trp Leu Phe Phe Asp Leu Asp Glu Asn Asn Lys Trp Lys Asp
    155 65                  70                  75                  80
    157 tat tgg gct ttt aaa gac aaa acc ccc gaa act gcg aca ata aca agg   288
    158 Tyr Trp Ala Phe Lys Asp Lys Thr Pro Glu Thr Ala Thr Ile Thr Arg
    159                 85                  90                  95
    161 aga tgg ctg ttc aga aaa caa aat aat ctg aaa aag gag act ttt gac   336
    162 Arg Trp Leu Phe Arg Lys Gln Asn Asn Leu Lys Lys Glu Thr Phe Asp
    163             100                 105                 110
    165 aat tta gtg aaa cta aca aag gga agc aag atg aga ttg tta ttc gat   384
    166 Asn Leu Val Lys Leu Thr Lys Gly Ser Lys Met Arg Leu Leu Phe Asp
    167         115                 120                 125
    169 ttg aat gcc gaa gtg agg act ggt tat gaa att gga aag aag atg aca   432
    170 Leu Asn Ala Glu Val Arg Thr Gly Tyr Glu Ile Gly Lys Lys Met Thr
    171     130                 135                 140
    173 tcc act tgg gat tca tcg gag gct gaa aag tta ttt aaa tat tgt gtg   480
    174 Ser Thr Trp Asp Ser Ser Glu Ala Glu Lys Leu Phe Lys Tyr Cys Val
    175 145                 150                 155                 160
    177 tca aaa ggt tac gga gac aat atc gat tgg gaa ctt gga aat gaa ccg   528
    178 Ser Lys Gly Tyr Gly Asp Asn Ile Asp Trp Glu Leu Gly Asn Glu Pro
    179                 165                 170                 175
    181 gac cac acc tca gct cac aat tta act gaa aag cag gtt gga gaa gat   576
    182 Asp His Thr Ser Ala His Asn Leu Thr Glu Lys Gln Val Gly Glu Asp
    183             180                 185                 190
    185 ttt aaa gca ctg cat aaa gtt cta gag aaa tat cca act ctt aac aag   624
    186 Phe Lys Ala Leu His Lys Val Leu Glu Lys Tyr Pro Thr Leu Asn Lys
    187         195                 200                 205
    189 gga tcg ctc gtt ggt cca gat gta ggg tgg atg ggc gtc agt wac gtc   672
W--> 190 Gly Ser Leu Val Gly Pro Asp Val Gly Trp Met Gly Val Ser Xaa Val
    191     210                 215                 220
    193 aag gga ttg gca gac gag gcr ggt gac cat gta ack gct ttt aca ctc   720
    194 Lys Gly Leu Ala Asp Glu Ala Gly Asp His Val Thr Ala Phe Thr Leu
    195 225                 230                 235                 240
    197 cac caa tat tat ttc gat gga aac acy tct gat gta tca ata tat ctt   768
    198 His Gln Tyr Tyr Phe Asp Gly Asn Thr Ser Asp Val Ser Ile Tyr Leu
    199                 245                 250                 255
    201 gat gcc aca tac ttt aag aag ctg caa caa cta ttt gat aaa gtg aaa   816
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RAW SEQUENCE LISTING DATE: 03/05/2003
PATENT APPLICATION: US/10/009,500B TIME: 15:15:15

Input Set : A:\Merck233.app
Output Set: N:\CRF4\03052003\J009500B.raw

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202 Asp Ala Thr Tyr Phe Lys Lys Leu Gln Gln Leu Phe Asp Lys Val Lys
203             260                 265                 270
205 gat gtt ttg aaa gat tct cca cat aaa gac gaa cca tta tgg ctt gga   864
206 Asp Val Leu Lys Asp Ser Pro His Lys Asp Glu Pro Leu Trp Leu Gly
207         275                 280                 285
209 gaa aca agt tct gga tac aac agc ggc aca gaa gat gta tcc gat cga   912
210 Glu Thr Ser Ser Gly Tyr Asn Ser Gly Thr Glu Asp Val Ser Asp Arg
211     290                 295                 300
213 tat gtt tca gga ttt cta aca tta gac aag ttg ggt ctc agt gca gcc   960
214 Tyr Val Ser Gly Phe Leu Thr Leu Asp Lys Leu Gly Leu Ser Ala Ala
215 305                 310                 315                 320
217 aac aat gta aag gtt gtt ata aga cag aca ata tac aat gga tat tat  1008
218 Asn Asn Val Lys Val Val Ile Arg Gln Thr Ile Tyr Asn Gly Tyr Tyr
219                 325                 330                 335
221 ggt ctc ctt gac aaa aac act tta gag ccg aat ccg gat tac tgg tta  1056
222 Gly Leu Leu Asp Lys Asn Thr Leu Glu Pro Asn Pro Asp Tyr Trp Leu
223             340                 345                 350
225 atg cat gtt cat aat tct ttg gtc gga aat aca gtt ttt aaa gtt gac  1104
226 Met His Val His Asn Ser Leu Val Gly Asn Thr Val Phe Lys Val Asp
227         355                 360                 365
229 gtt agt gat cca act aat aaa gca aga gtt tac gcg caa tgt acc aaa  1152
230 Val Ser Asp Pro Thr Asn Lys Ala Arg Val Tyr Ala Gln Cys Thr Lys
231     370                 375                 380
233 aca aat agc aaa cat act caa agc aga tat tac aag ggc tct ttg aca  1200
234 Thr Asn Ser Lys His Thr Gln Ser Arg Tyr Tyr Lys Gly Ser Leu Thr
235 385                 390                 395                 400
237 atc ttt gca ctt aat gtt gga gat gga gat gta acg tta aag atc ggt  1248
238 Ile Phe Ala Leu Asn Val Gly Asp Gly Asp Val Thr Leu Lys Ile Gly
239                 405                 410                 415
241 caa tac agc ggt aaa aaa att tat tca tac att ctg aca cct gaa gga  1296
242 Gln Tyr Ser Gly Lys Lys Ile Tyr Ser Tyr Ile Leu Thr Pro Glu Gly
243             420                 425                 430
245 gga caa ctt aca tca cag aaa gtt ctc ttg aat gga aag gaa ttg aac  1344
246 Gly Gln Leu Thr Ser Gln Lys Val Leu Leu Asn Gly Lys Glu Leu Asn
247         435                 440                 445
249 tta gtg tct gat cag tta cca gaa cta aat gca gat gaa tcc aaa aca  1392
250 Leu Val Ser Asp Gln Leu Pro Glu Leu Asn Ala Asp Glu Ser Lys Thr
251     450                 455                 460
253 tct ttc acc tta tcc cca aag aca ttt ggt ttt ttt gtt gtt tcc gat  1440
254 Ser Phe Thr Leu Ser Pro Lys Thr Phe Gly Phe Phe Val Val Ser Asp
255 465                 470                 475                 480
257 gct aat gtt gaa gca tgy aar aar                                  1464
258 Ala Asn Val Glu Ala Cys Lys Lys
259                 485
262 <210> SEQ ID NO: 3
263 <211> LENGTH: 488
264 <212> TYPE: PRT
265 <213> ORGANISM: Hirudinaria manillensis
267 <220> FEATURE:
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RAW SEQUENCE LISTING DATE: 03/05/2003
PATENT APPLICATION: **US/10/009,500B** TIME: 15:15:15

Input Set : **A:\Merck233.app**
Output Set: **N:\CRF4\03052003\J009500B.raw**

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268 <221> NAME/KEY: variation
269 <222> LOCATION: (223)
270 <223> OTHER INFORMATION: Tyr or Asn
272 <400> SEQUENCE: 3
273 Lys Glu Ile Ala Val Thr Ile Asp Asp Lys Asn Val Ile Ala Ser Ala
274   1               5                  10                  15
276 Ser Gly Ser Phe Leu Gly Val Ala Phe Asp Ala Ser Leu Phe Ser Pro
277              20                  25                  30
279 Lys Gly Leu Trp Ser Phe Val Asp Ile Thr Ser Pro Lys Leu Phe Lys
280          35                  40                  45
282 Leu Leu Glu Gly Leu Ser Pro Gly Tyr Phe Arg Val Gly Gly Thr Phe
283      50                  55                  60
285 Ala Asn Trp Leu Phe Phe Asp Leu Asp Glu Asn Asn Lys Trp Lys Asp
286  65                  70                  75                  80
288 Tyr Trp Ala Phe Lys Asp Lys Thr Pro Glu Thr Ala Thr Ile Thr Arg
289                  85                  90                  95
291 Arg Trp Leu Phe Arg Lys Gln Asn Asn Leu Lys Lys Glu Thr Phe Asp
292             100                 105                 110
294 Asn Leu Val Lys Leu Thr Lys Gly Ser Lys Met Arg Leu Leu Phe Asp
295         115                 120                 125
297 Leu Asn Ala Glu Val Arg Thr Gly Tyr Glu Ile Gly Lys Lys Met Thr
298     130                 135                 140
300 Ser Thr Trp Asp Ser Ser Glu Ala Glu Lys Leu Phe Lys Tyr Cys Val
301 145                 150                 155                 160
303 Ser Lys Gly Tyr Gly Asp Asn Ile Asp Trp Glu Leu Gly Asn Glu Pro
304                 165                 170                 175
306 Asp His Thr Ser Ala His Asn Leu Thr Glu Lys Gln Val Gly Glu Asp
307             180                 185                 190
309 Phe Lys Ala Leu His Lys Val Leu Glu Lys Tyr Pro Thr Leu Asn Lys
310         195                 200                 205
W--> 312 Gly Ser Leu Val Gly Pro Asp Val Gly Trp Met Gly Val Ser Xaa Val
313     210                 215                 220
315 Lys Gly Leu Ala Asp Glu Ala Gly Asp His Val Thr Ala Phe Thr Leu
316 225                 230                 235                 240
318 His Gln Tyr Tyr Phe Asp Gly Asn Thr Ser Asp Val Ser Ile Tyr Leu
319                 245                 250                 255
321 Asp Ala Thr Tyr Phe Lys Lys Leu Gln Gln Leu Phe Asp Lys Val Lys
322             260                 265                 270
324 Asp Val Leu Lys Asp Ser Pro His Lys Asp Glu Pro Leu Trp Leu Gly
325         275                 280                 285
327 Glu Thr Ser Ser Gly Tyr Asn Ser Gly Thr Glu Asp Val Ser Asp Arg
328     290                 295                 300
330 Tyr Val Ser Gly Phe Leu Thr Leu Asp Lys Leu Gly Leu Ser Ala Ala
331 305                 310                 315                 320
333 Asn Asn Val Lys Val Val Ile Arg Gln Thr Ile Tyr Asn Gly Tyr Tyr
334                 325                 330                 335
336 Gly Leu Leu Asp Lys Asn Thr Leu Glu Pro Asn Pro Asp Tyr Trp Leu
337             340                 345                 350
339 Met His Val His Asn Ser Leu Val Gly Asn Thr Val Phe Lys Val Asp
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RAW SEQUENCE LISTING ERROR SUMMARY DATE: 03/05/2003
PATENT APPLICATION: **US/10/009,500B** TIME: 15:15:16

Input Set : **A:\Merck233.app**
Output Set: **N:\CRF4\03052003\J009500B.raw**

Please Note:
Use of n and/or Xaa have been detected in the Sequence Listing. Please review the Sequence Listing to ensure that a corresponding explanation is presented in the <220> to <223> fields of each sequence which presents at least one n or Xaa.

Seq#:2; Xaa Pos. 223
Seq#:3; Xaa Pos. 223

VERIFICATION SUMMARY DATE: 03/05/2003
PATENT APPLICATION: **US/10/009,500B** TIME: 15:15:16

Input Set : **A:\Merck233.app**
Output Set: **N:\CRF4\03052003\J009500B.raw**

L:190 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:2 after pos.:672
L:312 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:3 after pos.:208